Absolute Risk of a Subsequent Abnormal Pap among Oncogenic Human Papillomavirus DNA-Positive, Cytologically Negative Women

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BACKGROUND. The addition of human papillomavirus (HPV) DNA testing to cytologic screening for cervical carcinoma is now being considered. The majority of women in screening cohorts who test positive for oncogenic types of HPV DNA have concurrent negative Pap tests. The absolute risk of a subsequent abnormal Pap test for these women is uncertain. Therefore, the proper counseling and clinical management of these women is also uncertain.

METHODS. A subcohort of 2020 women with a negative Pap test who tested positive at enrollment for oncogenic HPV DNA types using the Hybrid Capture 2 Test were followed for 57 months at Kaiser Permanente (Portland, OR). Absolute risks of new abnormal cytologic interpretations were computed using Kaplan–Meier methods. Logistic regression models were used to evaluate determinants of a new abnormal Pap test.

RESULTS. The cumulative incidence for a Pap test interpreted as atypical squamous cells or more severe (≥ ASC) was 16.8% (95% confidence interval [CI] = 15.0–18.6%), 6.4% (95% CI = 5.2–7.6%) for low-grade squamous intraepithelial lesions or more severe, and 2.2% (95% CI = 1.5–2.9%) for high-grade squamous intraepithelial lesions or more severe. By comparison, the cumulative incidence of greater than or equal to ASC among HPV-negative women was 4.2% (95% CI = 3.9–4.6%). The highest viral load (100 relative light units per the positive control or greater) was associated with a greater risk of an abnormal Pap test (odds ratio= 2.7, 95% CI = 1.7–4.1) than lower viral loads.

CONCLUSIONS. These results suggest that about 15% of women in annual screening programs who concurrently have a negative Pap test and a positive oncogenic HPV test will have a subsequent abnormal Pap test within 5 years. This risk estimate will be useful to the many clinicians and patients likely to be diagnosed with an HPV infection and negative cytology if HPV DNA is added to general screening. ***Cancer* 2002;95:2145–51.** *Published 2002 by the American Cancer Society.**
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Cervical carcinoma[1–3] and its immediate precursors[4] are causally related to cervical infection with 1 of 13 cancer-associated (oncogenic) human papillomavirus (HPV) types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68). Most HPV infections go unnoticed and regress. However, as the result of viral production, some may cause mild cytologic changes interpreted as atypical squamous cells (ASC) or low-grade squamous intraepithelial lesions (LSIL), which are commonly detected by standard Pap screening practices.

HPV DNA testing using the Hybrid Capture 2 Test (HC2; Digene, Gaithersburg, MD) is being proposed as a general population screening test in conjunction with the Pap test for women 30 years of age and older, as reassurance of the absence of high-grade cervical lesions or invasive carcinoma. Women with a concurrent negative Pap test and negative HC2 test have a substantially decreased risk of develop-

ing high-grade cervical lesions or cancer. However, most HPV DNA-positive women are negative for cytologic abnormalities. Clinicians and patients need to know the clinical significance of an HPV DNA-positive, cytologic-negative result with regard to prediction of subsequent cytologic abnormalities.

The absolute risk of developing an incident cytologic abnormality following an oncogenic HPV infection has not been described fully. In a study of 496 women younger than 22 years old attending a family planning clinic, Moscicki et al.[5] found that approximately 20% of participants with an (oncogenic or low-risk) HPV infection determined by polymerase chain reaction (PCR) were subsequently found to have a Pap test interpreted as LSIL during an intensive follow-up of 50 months with interval visits every 4 months.

In the current study, we estimated the absolute risk of developing an equivocal or definite cytologic abnormality among 2020 women participating in a general screening clinic at Kaiser Permanente Health Plan (Portland, OR) during a 57-month follow-up. The women were oncogenic HPV DNA-positive by HC2 and cytologically negative at their enrollment visit.

MATERIALS AND METHODS

Study Subjects

Between April 1, 1989 and November 2, 1990, 23,702 women were enrolled in a natural history study of HPV infection at the Kaiser Permanente prepaid health plan in Portland, OR as previously described.[4] The study was approved by Institutional Review Boards (IRB) of both the National Institutes of Health (NIH) and Kaiser Permanente. Consent was obtained from all participants in accordance with guidelines of the U.S. Department of Health and Human Services. Seven clinics (two health appraisal clinics and five obstetrics/gynecology clinics) performing Pap smears in Portland were used for recruiting women to the study. The cohort included approximately 50% of women undergoing cervical cytologic screening at Kaiser Permanente, which served approximately 25% of women residing in Portland when the study was initiated. Subjects were 16 years of age or older with a mean age of 35.9 (range, 16–94 years). Women were followed for up to 122 months and those with negative baseline smears had a median follow-up of longer than 6 years. A main analysis cohort of 20,810 women was established and followed passively as part of standard cytologic screening for cervical neoplasia. Women were excluded if they refused to participate (n = 1107), had undergone a hysterectomy (n = 1406), had an inadequate specimen for HPV testing (n = 195), had unsatisfactory or missing baseline cervical smears (n = 85), or had undergone a colposcopy rather than Pap smear screening at enrollment (n = 99).

Within this cohort, we identified a subcohort of 2511 women who tested positive for HPV DNA for oncogenic types (see below) and who were cytologically negative at baseline. For the purposes of this analysis, we further restricted participation to a group of 2020 women (80%) who had at least one additional visit after enrollment. Women in this subcohort had a median enrollment age of 28 years (range, 16–81 years).

Enrollment Examination

Subjects who consented as required by the IRBs at Kaiser Permanente and the NIH underwent a routine pelvic examination.[4] Exfoliated cervical cells were collected with an Ayre spatula and a cytobrush for Pap test screening. Next, a 10-mL sterile saline cervicovaginal lavage was performed on each subject to collect specimens for HPV testing. Amenable subjects completed either a written, self-administered questionnaire or a 20-minute telephone interview that assessed demographics, smoking habits, contraceptive practices at baseline, and parity. Questionnaire data were available for 1222 subjects (60.5%) in this analysis. Computerized records were reviewed to identify women who had a past medical history (cytologic or histologic) of cervical neoplasia or had been treated for cervical neoplasia before enrollment.

Pathology

Tests reported as "normal" or "benign reactive atypia" were reclassified as "negative for intraepithelial lesion or malignancy (negative)" according to the Bethesda 2001 classification.[6] Tests reported as "severe reactive atypia, possibly dysplasia" or "possible koilocytotic or condylomatous atypia" were classified as "atypical squamous cells (ASC)." Cytologic interpretations of dysplasia were reclassified as LSIL and high-grade squamous intraepithelial lesion (HSIL).

Follow-Up

During the study period, annual cytologic screening of women at Kaiser Permanente was standard practice. Tests were generally obtained at clinic visits if screening had not been performed within the previous 9 months or if there was clinical suspicion of a cervical abnormality. Patients with abnormal cytology were managed according to standard practice guidelines at Kaiser Permanente. Patients with histologic diagnoses of cervical intraepithelial neoplasia grade 2 (CIN2) or more severe and some CIN1 from colposcopically directed biopsies were treated by ablative or excisional therapy. HPV testing was not used to direct patient management. Women who had no evidence of cyto-

logic abnormalities during follow-up had a median of two follow-up Pap tests and women with a diagnosis of ASC or more severe cytology during follow-up had a median of four follow-up Pap tests (range, 1–12) within 57 months.

HPV Testing

Frozen aliquots of lavages were tested for HPV DNA by HC2 using probe B, an assay for the DNA detection of 13 cancer-associated HPV types.[7] Signal strengths in relative light units (RLU) were compared to 1 pg/mL HPV type 16 DNA-positive controls (RLU/PC).Specimens with greater than or equal to 1 RLU/PC were considered HPV DNA positive. Log units of the RLU/PC values greater than or equal to 1 (1–10, 10–100, ≥ 100 RLU/PC) were used as semiquantitative measures of viral load among HPV DNA-positive women. HC2 performed at Digene was masked to the clinical results.

Analysis

Follow-up time was divided into an initial period of 9 months to capture prevalent disease followed by analogous yearly intervals (e.g., 9–21 months, 21–33 months) to the completion of the study for a total time of 122 months of follow-up. The 57-month follow-up period was chosen for comparability to a study of incident LSIL.[5] Functionally, it represents the screening that occurred up to the fourth year of annual screening.

Cumulative incidence rates for the first Pap tests interpreted either as ASC or more severe, LSIL or more severe, and HSIL or more severe among oncogenic HPV DNA-positive women and oncogenic HPV-negative women were calculated using Kaplan–Meier methods. A threshold of ASC or more severe was used for examining determinants of a subsequent cytologic abnormality. This is because of recent evidence to suggest that for the risk of progression to high-grade cervical lesions for women who are HPV DNA positive, cytologic ASC is indistinguishable from risk for women who have LSIL cytology.[8] Under new guidelines, women who are HPV positive and have an ASC Pap test will be managed the same as if they had an LSIL Pap test.[6] Stratified analyses were used to assess crude associations of covariates with the prospective risk of an abnormal Pap (≥ ASC). Variables that were associated with outcome were included in the multivariate model; smoking was also included in the multivariate model based on a previous reported association.[5]

In addition to the longitudinal analysis, we evaluated the association of covariates with prospective risk of cytologic abnormalities using a multivariate model to control for potential confounding by screening patterns. Conditional logistic regression was used to calculate odds ratios (OR) with 95% confidence intervals (95%CI) as an estimate of relative risk (RR). Cases of ASC or more severe (n = 289, n = 163 with a questionnaire) were matched to controls by number of Pap tests during follow-up, time from enrollment to the last visit with a normal test (± 3 months), and time from enrollment to the visit with cytologic abnormality (± 3 months). Each case was matched to a median of 223 controls (range, 2–779) and each control was matched to a median of 61 cases (range, 1–90). In a subset of cases and controls with questionnaires, each case was matched to a median of 163 controls (range, 2–482) and each control was matched to a median of 35 cases (range, 1–55). To test for statistically significant dose-response relationships, covariates were treated as continuous variables and tested as to whether the resulting slope (β coefficient) was nonzero.

RESULTS

Women with negative cytology and a positive test for oncogenic HPV DNA had a crude cumulative incidence of 16.8% (95% CI = 15.0–18.6%) for ASC or more severe, 6.4% (95% CI = 5.2–7.6%) for LSIL or more severe, and 2.2% (95% CI = 1.5–2.9%) for HSIL or more severe (Fig. 1A). By comparison, women with negative baseline tests and a negative test for oncogenic HPV DNA at enrollment had a crude cumulative incidence for ASC or more severe of 4.2% (95% CI = 3.9–4.6%), for LSIL or more severe of 1.1% (95% CI = 0.9–1.3%), and for HSIL or more severe of 0.3% (95% CI = 0.2–0.4%; Fig. 1B). The corresponding estimated RRs for ASC or more severe, LSIL or more severe, and HSIL or more severe associated with an oncogenic HPV DNA-positive test compared with an HPV DNA-negative test were 4.0, 5.8, and 7.3, respectively.

In a stratified analysis of the crude incidence, women were at a higher risk of subsequent ASC or more severe cytology in this subcohort if they were screened more often, were younger, had higher viral loads, or were current barrier contraceptive users (Table 1). Women with five or more Pap tests during follow-up had an almost fourfold greater 57-month cumulative incidence of ASC or more severe (29.8%; 95% CI = 26.2–33.4%) than women with two Pap tests (7.9%; 95% CI = 4.9–10.8%). Women younger than 22 had a threefold greater cumulative incidence of ASC or more severe (26.6%; 95% CI = 22.0–31.2%) than women 40 years of age and older (8.3%; 95% CI = 5.5–11.0%). Women with the highest viral load (≥ 100 RLU/PC) had a 2.5-fold greater 57-month cumulative incidence of ASC or more severe (29.5%; 95% CI = 23.9–35.1%) than women with the lowest viral load (1–10 RLU/PC; 12.0%; 95% CI 10.0–14.0%). Conversely, women with three or more live births (6.8;

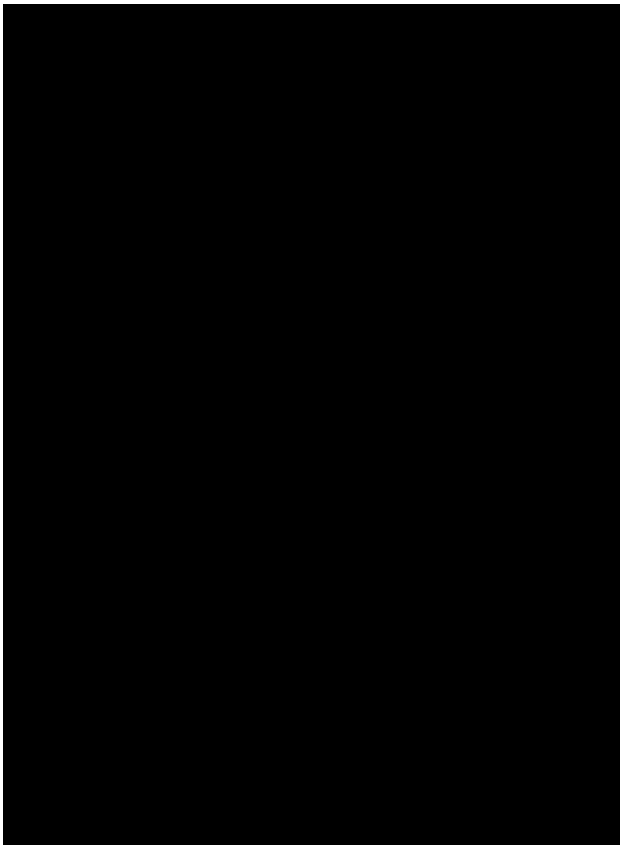

FIGURE 1. Cumulative incidence rates for cytologic abnormalities (atypical squamous cells or more severe) in a 57-month follow-up among baseline oncogenic HPV DNA-positive (A) and HPV DNA-negative women (B) without baseline cytologic abnormalities. Bars indicate 95% confidence intervals.

95%CI = 3.1–10.5%) were less likely to get a future ASC or more severe than nulliparous women. Among oncogenic HPV-negative women, those who were younger, had a past medical history of cervical dysplasia, or were screened more often were more likely to receive a ASC or more severe Pap during follow-up (Table 1).

We next examined the effects of the aforementioned covariates in conditional logistic models, matching on screening patterns (including the number of Pap tests) to control for their effects, for the detection of ASC or more severe among the oncogenic HPV DNA-positive women. In an adjusted model for ASC or more severe (which included viral load, smoking habits, age, use of barrier methods at baseline, and parity), viral load was a strong determinant of risk (Table 2). The risk of ASC or more severe increased monotonically with increasing level of viral load ($P_{\text{Trend}} < 0.001$). The risk was significantly elevated for viral loads of 10–100 RLU/PC (OR = 2.4, 95% CI = 1.6–3.5) and for viral loads of 100 RLU/PC or higher (OR = 2.7, 95% CI = 1.7–4.1). Smoking one or more packs per day (OR = 1.8, 95% CI = 1.1–3.0) and current use of barrier contraceptive methods (OR = 1.4, 95% CI = 0.96–2.1) were marginally associated with an increased risk of ASC or more severe. Three or more lifetime births (OR = 0.53, 95% CI = 0.26–1.1) were marginally associated with a decreased risk of ASC or more severe. Age was no longer associated with ASC or more severe. These risk estimates did not change appreciably when a threshold of LSIL was used for the detection of cytologic abnormalities or when restricted to women older than 30 (data not shown).

DISCUSSION

This study indicates that about 17% of women attending a general U.S. screening clinic with a negative Pap test and a positive HPV DNA test for oncogenic types by the HC2 test will develop an abnormal Pap (≥ ASC) within approximately 5 years. The incidence of abnormal cytology was highly dependent on the number of screening visits. Therefore, screening recommendations will impact on the estimate. For women 30 years and older for whom HPV DNA testing by HC2 is being proposed, 11.1% (95%CI = 8.9–13.2%) of oncogenic HPV DNA-positive women will have an ASC or more severe Pap within 57 months compared with 3.2% (95%CI = 2.9–3.6%) of oncogenic HPV DNA-negative women. Large differences in incidence curves and crude determinants of ASC or more severe between oncogenic HPV DNA-positive and HPV DNA-negative groups suggest that each represents a significantly different risk group.

A diagnostic threshold of ASC for cytologic abnormality was chosen because there is virtually no differential risk of progression to high-grade neoplasia for HPV-infected women with an ASC Pap compared with women with an LSIL Pap,[8] suggesting that these interpretations are biologically indistinguishable. Inclusion of ASC resulted in a 2.5-fold increase in the rate of cytologic abnormalities compared with an LSIL threshold (16.8% vs. 6.4%). The rate for subsequent LSIL was lower in this more broadly representative population with annual screening than the rates reported recently in a high-risk younger population with active follow-up visits occurring every 4 months.[5]

In this study, the baseline HPV DNA test was used as a marker of risk for a subsequent abnormal Pap. It is likely that some subsequent Pap tests called ASC, and even a few LSIL, were not due to any HPV infection, particularly in older women. They were a consequence of age-related atrophic changes in the cervical epithelia that mimic equivocal cytologic changes. Therefore, we may have overestimated the number of subsequent abnormalities due to oncogenic HPV alone. The primary aim of this analysis was to evaluate the relationship of a single HPV test to future cytologic

TABLE 1
Comparisons of Cumulative Incidence Rates for an Abnormal Pap Test (≥ ASC) among Women Who Are Cytologically Negative and Tested Either Positive or Negative for an Oncogenic Type of HPV at Enrollment

	Oncogenic HPV DNA positive		Oncogenic HPV DNA negative	
Characteristics	***n*[a]**	**Cumulative incidence (95% CI)**	***n*[a]**	**Cumulative Incidence (95% CI)**
Overall	2020	16.8 (15.0–18.6)	14,606	4.2 (3.9–4.6)
Age (yrs)				
≥ 40	436	8.3 (5.5–11.0)	5646	3.0 (2.5–3.4)
30–39	450	13.9 (10.4–17.3)	4690	3.6 (3.0–4.2)
22–29	663	18.7 (15.3–22.1)	3143	5.0 (4.2–5.9)
< 22	471	26.6 (22.0–31.2)	1127	13.0 (10.6–15.4)
Viral load (RLU/PC)				
1–10	1216	12.0 (10.0–14.0)		
10–100	507	21.2 (17.2–25.1)		
≥ 100	297	29.5 (23.9–35.1)		
Past medical history				
No	1677	16.7 (14.7–18.6)	12,685	3.7 (3.4–4.1)
Yes	343	17.5 (13.1–21.9)	1921	7.2 (6.0–8.4)
No. of Pap smears				
2	541	7.9 (4.9–10.8)	3778	1.7 (1.1–2.2)
3–4	837	9.9 (7.7–12.0)	6483	2.8 (2.3–3.2)
≥ 5	642	29.8 (26.2–33.4)	4345	7.5 (6.7–8.3)
Smoking				
Never	678	13.8 (11.0–16.6)	6314	3.4 (2.9–3.8)
Former	243	16.9 (11.8–22.0)	2253	3.8 (3.0–4.6)
< 1 pack/day	173	18.2 (11.9–24.5)	819	4.5 (2.9–6.0)
≥ 1 pack/day	120	17.0 (9.7–24.3)	706	5.2 (3.4–7.0)
No. of live births				
0	512	19.7 (15.9–23.5)	2736	4.0 (3.2–4.8)
1–2	509	14.3 (11.0–17.6)	4830	3.3 (2.8–3.9)
≥ 3	192	6.8 (3.1–10.5)	2541	4.0 (3.2–4.8)
Current OC use				
No	841	14.7 (12.1–17.2)	8452	3.5 (3.1–3.9)
Yes	356	17.7 (13.2–22.2)	1542	5.0 (3.7–6.2)
Current barrier contraceptive use				
No	984	14.3 (12.0–16.7)	8613	3.6 (3.2–4.0)
Yes	213	20.9 (14.9–27.0)	1381	4.3 (3.2–5.5)
Income ($)				
< 20K	398	16.9 (12.8–20.9)	1892	5.0 (3.9–6.1)
20–50K	606	15.6 (12.5–18.7)	5696	3.8 (3.2–4.3)
≥ 50 K	169	11.9 (6.7–17.1)	2064	2.5 (1.8–3.3)

ASC: atypical squamous cells; HPV: human papillomavirus; RLU/PC: relative light units per positive control; CI: confidence interval; OC: oral contraceptive.
[a] Number of women at baseline.

abnormalities in the context of general screening. Therefore, only a single cytologic interpretation was used, as occurs in standard clinical practice. Histologic outcomes were not used. Histologic diagnoses of mild abnormalities (i.e., CIN1) are only moderately reproducible[10] and do not represent a significantly better measure of mild abnormalities than cytologic interpretation.

The acquisition of new HPV infections also may have contributed to the rates we observed. A subset of 105 women in the subcohort who had an abnormal Pap (≥ ASC) within the 57 months were also tested twice for HPV DNA by MY09/M11 L1 consensus primer PCR within a larger incident case–control study.[11] Overall, 65% of these women (n = 68) had at least one of the same oncogenic types in both tests. This percentage decreased to 48% (22 of 46 women) among women with a follow-up time greater than the median follow-up time of 512 days (range, 59–1650 days).

In our multivariate analysis, we found that a high viral load was a significant risk factor for subsequent cytologic abnormalities. In contrast, women with low viral load infections had a very low risk of cytologic abnormalities. We also did not find viral load to be a risk factor for histologic CIN3 and cancer, although extremely low viral loads may have a relatively low risk of future high-grade disease.[12] However, it is unlikely

TABLE 2
OR and Corresponding 95% CI for ≥ ASC Associated with Risk Factors in a Conditional Logistic Model Matching Cases and Controls on Screening Behavior

	All (n = 288)	Answered qustionnaire (n = 163)	
Risk factors	**Unadjusted OR (95% CI)**	**Unadjusted OR (95% CI)**	**Adjusted OR (95% CI)**
Viral load (RLU/PC)			
1–10	1	1	1
10–100	1.8 (1.3–2.3)	2.7 (1.9–3.8)	2.4 (1.6–3.5)
≥ 100	2.9 (2.2–3.9)	2.9 (1.9–4.4)	2.7 (1.7–4.1)
	$P_{Trend} < 0.001$	$P_{Trend} < 0.001$	$P_{Trend} < 0.001$
Age (yr)			
≥ 40	1	1	1
30–39	1.6 (1.0–2.5)	1.4 (0.87–2.3)	1.2 (0.70–2.0)
22–29	1.9 (1.2–2.8)	1.8 (1.2–2.9)	1.3 (0.75–2.1)
< 22	3.0 (2.0–4.5)	2.4 (1.5–4.0)	1.4 (0.79–2.5)
	$P_{Trend} < 0.001$	$P_{Trend} = 0.001$	$P_{Trend} = 0.2$
Past history of dysplasia			
No	1	1	
Yes	0.87 (0.63–1.2)	1.1 (0.76–1.7)	
Smoking (cigarettes)			
Never		1	1
Former		1.3 (0.86–1.9)	1.3 (0.88–2.0)
< 1 pack/day		1.4 (0.93–2.3)	1.2 (0.74–1.9)
≥ 1 pack/day		1.6 (0.93–2.6)	1.8 (1.1–3.0)
		$P_{Trend} = 0.03$	$P_{Trend} = 0.04$
Currently using barrier methods			
No		1	1
Yes		1.5 (1.1–2.2)	1.4 (0.96–2.1)
Past no. of live births			
0		1	1
1–2		0.80 (0.57–1.1)	0.94 (0.66–1.4)
≥ 3		0.39 (0.21–0.73)	0.53 (0.26–1.1)
		$P_{Trend} = 0.002$	$P_{Trend} = 0.10$

OR: odds ratio; CI: confidence interval; ASC: atypical squamous cells; RLU/PC: relative light units per positive control
[a] Number of cases of ASC or more severe.

that viral load measurements will be clinically useful for predicting future CIN3 (precancer) and cancer among cytologic-negative women because of the significant overlap in viral loads for any cytologic outcome.

Smoking, after adjusting for screening and other covariates, was marginally associated with the risk of ASC or more severe. Similarly, one study found that smoking increased the risk for the development of subsequent LSIL among 496 HPV-infected women attending family planning clinics.[5] The authors from that study suggested that HPV infection and LSIL represent discrete stages in the multistage pathogenesis of cervical carcinoma. Another explanation for the increase risk for cytologic abnormalities associated with smoking is that smokers, in contrast to multiparous women, engage in higher-risk sexual behavior compared with all HPV DNA-positive women and are more likely to acquire new HPV infections during follow-up. Alternatively, smoking and parity could modify the cervical epithelia in a manner as to increase and decrease, respectively, the "detectability" of these cytologic abnormalities. However, there is evidence that argues against such an interpretation for the effect of multiparity. First, data suggest an increased detectability of oncogenic HPV infection[13] and cytologic abnormalities[14] during pregnancy. Second, increased parity has been associated with a prolonged presence of the transformation zone on the exocervix in older women,[15] which could increase the likelihood of subsequent infection.

We conclude that about one of every six women with a positive test for oncogenic HPV DNA by HC2 will develop a detectable cytologic abnormality (≥ ASC) within 5 years in a typical clinical setting with yearly screening. Estimating the risk of subsequent cytologic abnormalities in women who test positive for HPV and negative for cytology has important implications if HPV testing in conjunction with cytologic screening is adopted for primary screening. The com-

plicated issue of combining HPV testing with cytologic screening was not addressed in this analysis. Whether this absolute risk (equivalent to about 15% positive predictive value) justifies subsequent yearly follow-up is a clinical issue worth studying in a formal cost-utility analysis, which will require future analyses of these and other data. We emphasize that women who were Pap screened more frequently were much more likely to be diagnosed with cytologic abnormalities (Table 1) and that screening intensity will significantly influence absolute risk estimates of abnormal Pap tests. Viral load was the most significant (nonscreening) determinant for the future risk of mild cytologic abnormalities. This is consistent with current biologic understanding that productive viral infections cause the cytomorphologic abnormalities interpreted as mildly abnormal Pap tests. However, only a minority of HPV infections became microscopically apparent as measured by standard cytologic screening practices and fewer still manifested as cytologic HSIL to indicate underlying high-grade cervical neoplasia.

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